Supplementary information

Antibacterial 45S5 Bioglass®-based scaffolds reinforced with genipin cross-linked gelatin for bone tissue engineering

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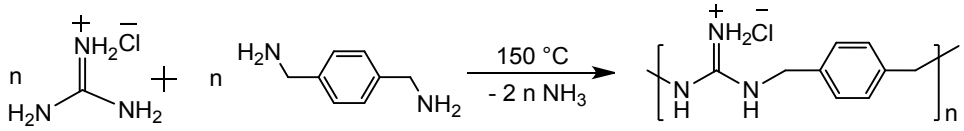
Poly(*p*-xylyleneguanidine) hydrochloride (PPXG) synthesis and structural characterization

Synthesis and NMR characterization

Poly(*p*-xylyleneguanidine) hydrochloride (PPXG) was made by condensation polymerization according to the scheme 1.



guanidine hydrochloride	[95.54]	0.050 mol	6.18 g	1 eq
p-xylylenediamine	[136.19]	0.050 mol	4.78 g	1 eq

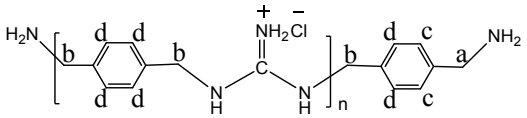
Scheme 1: Synthetic scheme for the formation of PPXG.

The polymer was structurally characterized using NMR. ^{1}H- (300 MHz) and ^{13}C- (75 MHz) NMR spectra were recorded on a Bruker Ultrashied-300 spectrometer in MeOD. The peaks were assigned as follows:

^{1}H-NMR: 300 MHz, MeOD; δ (ppm) = 3.81(s, 2H, (*CH*$_2$)NH$_2$) 4.45 (m, 2H, NH*CH*$_2$*C*$_6$*H*$_5$); 7.02 (m, Ar-*H*); 7.35 (m, Ar-*H*).

^{13}C-NMR: 75 MHz, MeOD; δ (ppm) = 45.56 (s, NH*CH*$_2$C$_6$H$_5$); 46.13 (s, *(CH*$_2$*)*NH$_2$); 128.81, 137.55 (m, Ar-*C*); 157.64, 158.64 (s, *C*=NH).

^{1}H-^{13}C correlation experiments were conducted on a Bruker Avance 600 spectrometer with a 5 mm multinuclear gradient probe at 25 ℃ using MeOD as solvent. 2D NMR spectrum heteronuclear single quantum coherence (HSQC) was used to assign peak positions in ^{13}C-NMR as shown in Figure S1.



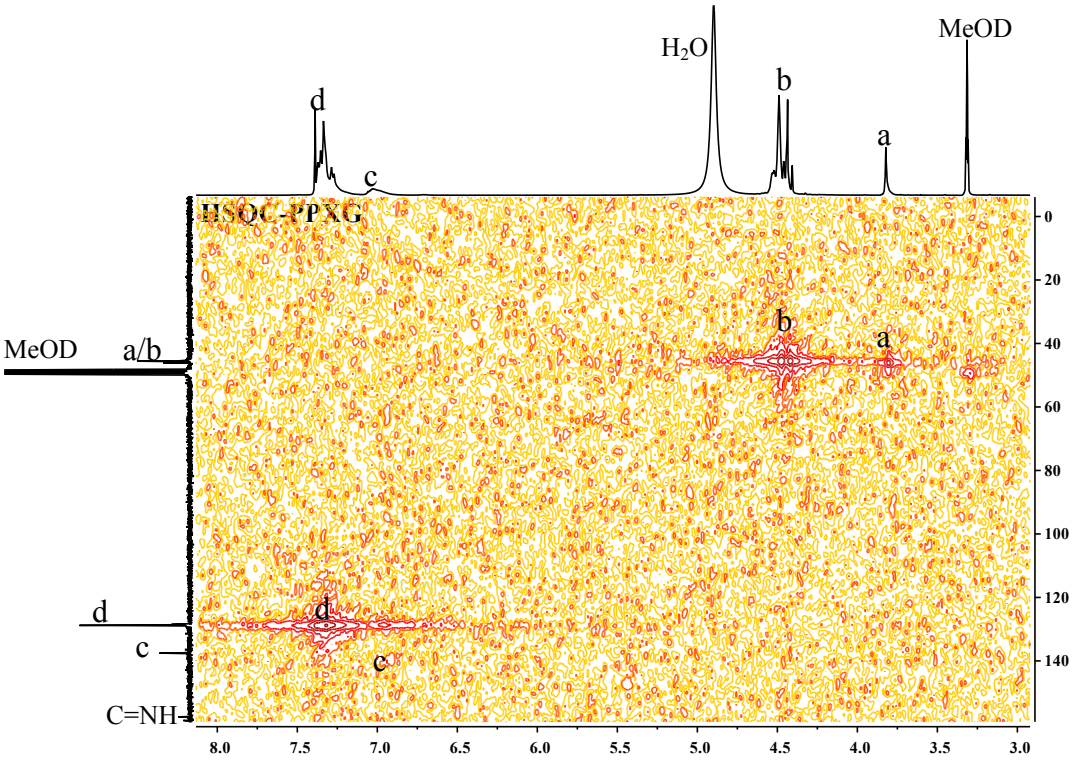


Figure S1. 2D ^{1}H-^{13}C HSQC NMR spectrum of PPXG in MeOD.

APCI analysis

APCI-mass spectrum was recorded on a Thermo Fisher Scientific Finnigan LTQ-FT spectrometer. The sample was dissolved in methanol. APCI-mass spectra were used to confirm the chain ends of PPXG (Figure S2). Four different types of chain structures were found i.e. PPXG chains with one guanidine and one amino group (structure A), guanidine and amino groups at both chain-ends (structures B and C) and ring structure without any chain-ends (structure D). No attempts were made to separate different structures and the sample was used as such for antibacterial tests and coating of scaffolds.

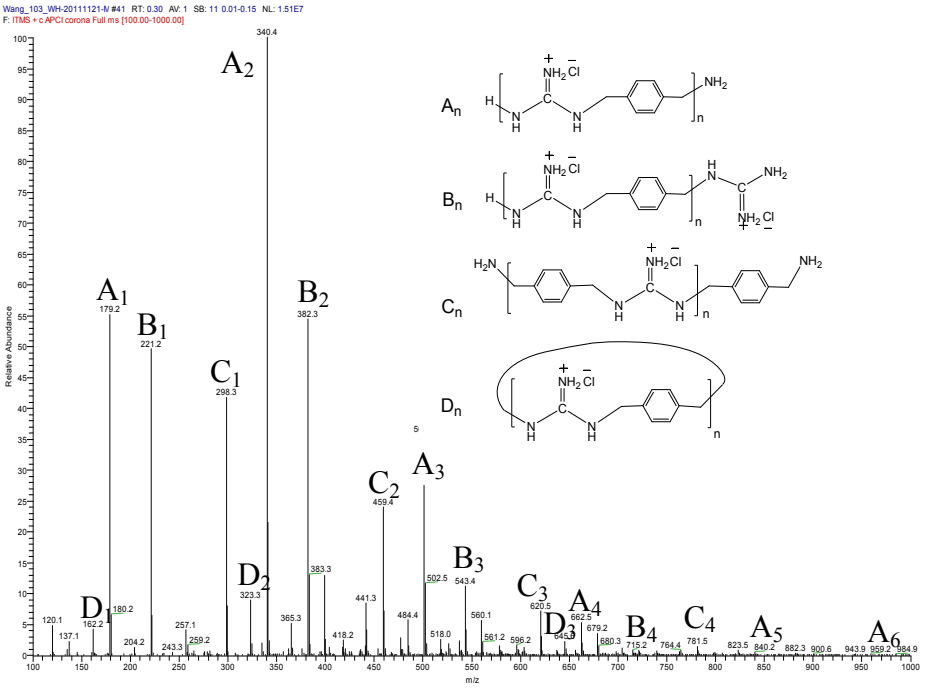


Figure S2. APCI-Spectrum of PPXG.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF-MS) analysis

MALDI-TOF MS was used for determination of molecular weight of PPXG. Bruker Reflex III apparatus equipped with a N_2 laser (λ = 337 nm) in linear mode at an acceleration voltage of 20 kV was used. Indole-3-acetic acid (IAA, Fluka, 99.0%) was used as a matrix material. Samples were prepared with the dried droplet method from Methanol solution by mixing matrix und polymer in a ratio of 20: 5 (v/v) and applying approximately 1 μL to the target spot.

The molecular weight of the PPXG determined by MALDI-TOF MS was M_n: 2200, M_w: 2500 and PDI: 1.12.

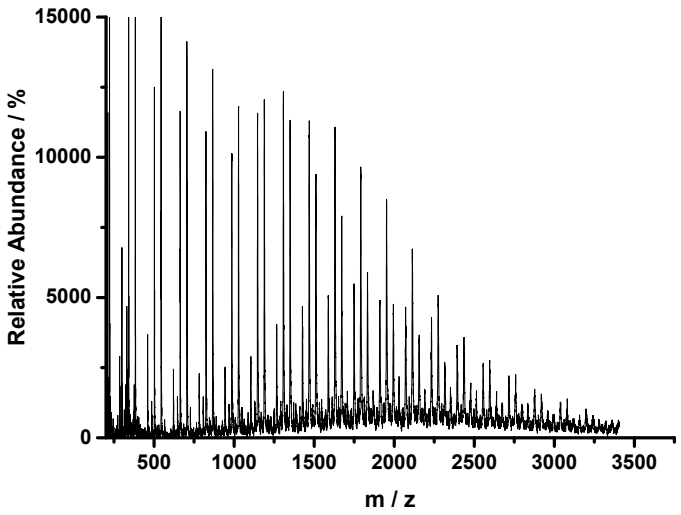


Figure S3. MALDI-ToF-MS-Spectrum of PPXG.

Thermal characterization

Thermal Analysis was performed on Mettler Toledo thermal analyzers comprising 821 DSC and 851 TG modules. By recording thermogravimetric (TG) traces in nitrogen atmosphere with a flow rate of 60 mL · min^{-1}, the thermal stability was determined; a sample size of 12 ± 2 mg and a heating rate of 10 K · min^{-1} was used for each measurement. The temperature of thermal decay (T_d) was taken as the inflection point of the TG curve. Differential scanning calorimetry (DSC) was performed in nitrogen atmosphere (flow rate 80 mL · min^{-1}) with a heating rate of 20 K · min^{-1}; the inflection point of the baseline in the second heating cycle was taken as glass transition temperature (T_g).

PPXG showed high glass transition temperature (T_g =150 ^oC; Figure S4) .Thermogravimetric analysis (Figure S5) showed, that the significant mass loss (85%) took only after 350^oC thereby showing high thermal stability.

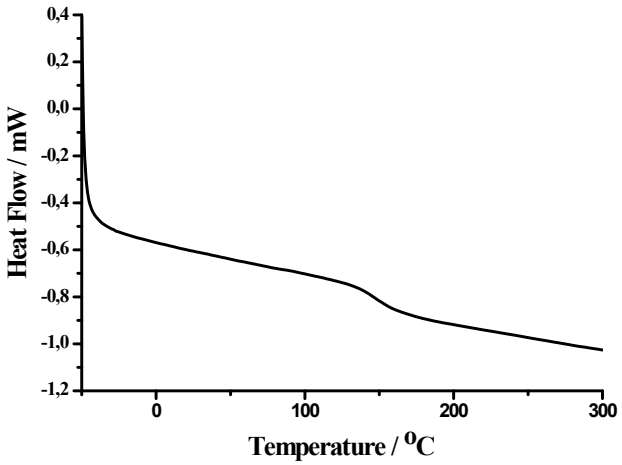


Figure S4. Differential scanning calorimetric analysis of PPXG showing glass transition temperature at 150°C.

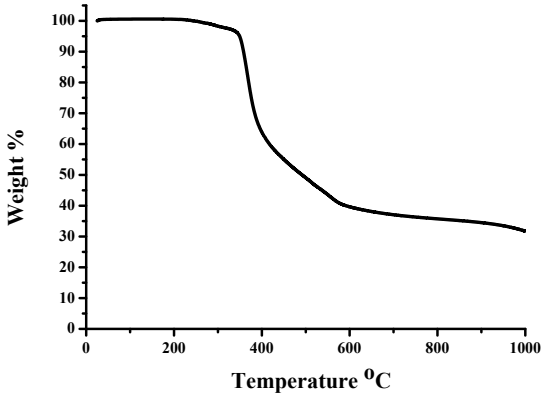


Figure S5. Weight loss vs. temperature curve for PPXG.

Antibacterial Test (MIC and MBC Test)

E.coli (Gram negative)

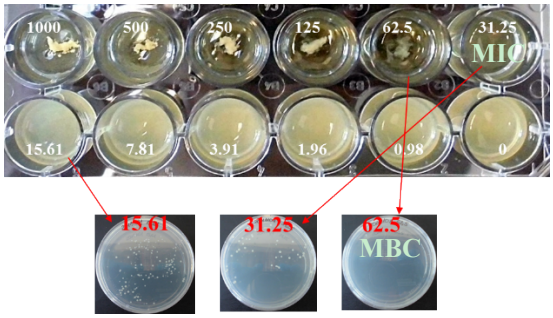


Figure S6. Photographs of MIC and MBC test with *E.coli* as test organism.

B.Subtilis (Gram positive)

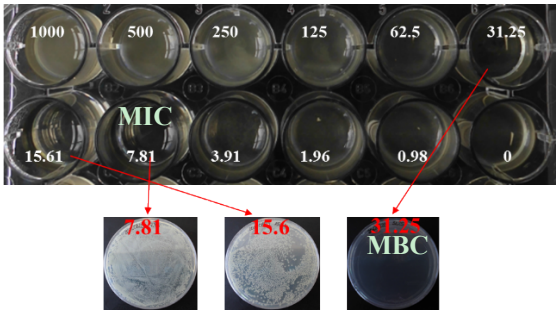


Figure S7. Photographs of MIC and MBC test with *B.Subtilis* as test organism.